Genetic Variants of DNA Repair Genes and Prostate Cancer: A Population-Based Study

Jamie D. Ritchey,[1] Wen-Yi Huang,[2] Anand P. Chokkalingam,[3] Yu-Tang Gao,[4] Jie Deng,[4] Paul Levine,[1] Frank Z. Stanczyk,[5] and Ann W. Hsing[2]

[1]George Washington University School of Public Health and Health Services, Washington, District of Columbia; [2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland; [3]Celera Diagnostics LLC, Alameda, California; [4]Shanghai Cancer Institute, Shanghai, China; and [5]Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, California

Abstract

As part of a population-based case-control study in Shanghai, China, we investigated whether variants in several DNA repair genes, either alone or in conjunction with other risk factors, are associated with prostate cancer risk. Genomic DNA from 162 patients newly diagnosed with prostate cancer and 251 healthy men randomly selected from the population were typed for five nonsynonymous DNA repair markers. We found that the *XRCC1*-Arg399Gln AA and the *MGMT*-Leu84Phe CT+TT genotypes were associated with an increased risk of prostate cancer [odds ratio (OR), 2.18; 95% confidence interval (CI), 0.99-4.81 and OR, 1.99; 95% CI, 1.19-3.34, respectively]. In contrast, *XRCC3*-Thr241Met, *XPD*-Lys751Gln, and *MGMT*-Ile143Val markers showed no significant associations with risk, although due to the much lower frequency of their variant alleles in this population we cannot rule out small to modest effects. There was a significant interaction between the *MGMT-84* marker and insulin resistance ($P_{interaction}$ = 0.046). Relative to men with the *MGMT-84* CC genotype and a low insulin resistance (<0.097), those having the CT-TT genotype and a greater insulin resistance had a 5.4-fold risk (OR, 5.39; 95% CI, 2.46-11.82). In addition, for the *XRCC3-241* marker, relative to men with the CC genotype and a low intake of preserved foods (<12.7 g/d), those harboring the CT+TT genotype and having a higher intake of preserved foods (>12.7 g/d), which contain nitrosamines and nitrosamine precursors, had a significantly increased risk of prostate cancer risk (OR, 2.62; 95% CI, 1.13-6.06). In contrast, men with the CT+TT genotype and a low intake of preserved foods had a 69% reduction in risk (OR, 0.31; 95% CI, 0.10-0.96; $P_{interaction}$ = 0.005). These results suggest that genetic variants in the DNA repair pathways may be involved in prostate cancer etiology and that other risk factors, including preserved foods and insulin resistance, may modulate prostate cancer risk in combination with genetic susceptibility in these repair pathways. Replication in larger studies is necessary to preclude chance findings, particularly those among subgroups, and clarify the mechanisms involved. (Cancer Epidemiol Biomarkers Prev 2005;14(7):1703–9)

Introduction

Prostate cancer is the most common cancer diagnosed among men in the United States. In 2005, an estimated 232,090 new cases will be diagnosed and 30,350 men will die from prostate cancer in the United States (1). African-Americans have the highest reported rates of prostate cancer in the world, with an age-adjusted rate of 137 per 100,000, which is 60 times higher than the reported incidence in Shanghai, China, where the reported incidence is the lowest in the world at 2 per 100,000 (2, 3). Reasons for the large racial differences in prostate cancer risk are unclear.

Although several factors have been implicated in prostate cancer etiology, only age, race, and a family history of prostate cancer are established risk factors. Data from previous twin and family studies suggest a genetic component for prostate cancer (4). To date, six rare, highly penetrant loci have been identified; however, these loci are estimated to be responsible for only 5% to 10% of all prostate cancer cases (5, 6). More common polymorphisms, such as those in DNA repair genes, may account for a larger proportion of prostate cancer in the population despite having a lower penetrance and conferring a much lower prostate cancer risk on individuals (7, 8).

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Requests for reprints: Ann W. Hsing, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, EPS-MSC 7234, 6120 Executive Boulevard, Bethesda, MD 20892-7234. Phone: 301-496-1691; Fax: 301-402-0916. E-mail: hsinga@mail.nih.gov

Genomic stability and integrity are important in maintaining accurate DNA replication. DNA disruptions can lead to gene rearrangements, translocations, amplifications, and deletions, which can in turn contribute to cancer development (9, 10). DNA is assaulted on a daily basis by a variety of endogenous processes and exogenous factors, including UV light, cigarette smoke, dietary factors, reactive oxygen species, and carcinogens, all of which can cause varying degrees of DNA damage and lead to DNA mutation (11). DNA repair mechanisms defend against these exogenous insults, correcting DNA damage as well as normal replication errors.

There are a number of DNA repair pathways, each responsible for repairing a different type of DNA damage. Base excision repair removes simple base modifications, including single-strand breaks, oxidative DNA damage, and alkylation and nonbulky adducts (12). Nucleotide excision repair removes larger lesions, which often result from environmental damage, including UV radiation and external carcinogens (13). Alkyltransferases directly reverse DNA damage by transferring alkyl groups from damaged DNA onto the transferase enzyme (14). Double-stranded DNA breaks are repaired through mechanisms including the homologous recombination repair pathway (15).

To clarify further the role of DNA repair pathways in prostate carcinogenesis, we investigated common nonsynonymous single nucleotide polymorphisms (SNP) in four DNA repair genes in a population-based case-control study in Shanghai, China. These genes, all previously studied in relation to other malignancies, include the X-ray repair cross-complementary group 1 (*XRCC1*)-Arg399Gln (G to A),

involved in base excision repair; the *XRCC3*-Thr241Met (C to T), involved in homologous recombination repair; the O^6-methylguanine-DNA-methyltransferase (*MGMT*)-Ile143Val (A to G) and *MGMT*-Leu84Phe (C to T), involved in direct damage reversal; and the xeroderma pigmentosum group D (*XPD*)-Lys751Gln (A to C), involved in nucleotide excision repair (16–18).

We also examined the combined effects of these DNA repair variants with a number of risk factors that have been reported in this study population. For example, in earlier reports, we have shown that abdominal obesity (measured by waist-to-hip ratio), insulin resistance (measured by the ratio of fasting serum insulin to glucose levels), and consumption of preserved foods are associated with a significant excess risk of prostate cancer in Chinese men and that consumption of allium vegetables is associated with a lower risk (19–21). Obesity and insulin resistance are closely linked to chronic inflammation, which may result in DNA damage (22, 23). Salted and preserved foods contain nitrosamines and nitrosamine precursors that induce DNA damage that is mitigated by DNA repair mechanisms (22, 23). Also, organosulfur compounds in allium vegetables may directly modulate DNA repair capacity (24). Thus, in this report, we evaluated the individual and combined effect of DNA repair allelic variants with these risk factors on prostate cancer risk.

Materials and Methods

Study Population. Details of this population-based case-control study have been reported previously (19, 20). Briefly, cases were permanent residents of Shanghai newly diagnosed with prostate cancer (ICD-9 185) between 1993 and 1995, identified through a rapid reporting system in 28 collaborating hospitals in urban Shanghai. Cases had no previous history of cancer. Four cases were excluded from the study after Chinese and American pathologists jointly confirmed them to be benign prostatic hyperplasia. The rapid reporting system captured over 95% of the cases diagnosed in urban Shanghai during the study period. Based on records maintained at the Shanghai Resident Registry, male controls with no history of cancer were selected at random from the 6.5 million permanent Shanghai residents over 18 years of age and frequency matched to cases by age (5-year intervals). This study was approved by the Institutional Review Boards at the National Cancer Institute, the Shanghai Cancer Institute, and the George Washington University School of Public Health and Health Services.

Interview. Using a structured questionnaire, trained personnel conducted in-person interviews to collect information on demographic characteristics, diet and smoking history, consumption of alcohol and other beverages, medical history, family cancer history, physical activity, and sexual behavior. Diet was assessed by means of a 122-item food frequency questionnaire developed for and validated in the Shanghai population. Based on the 122 food items, over 40 food groups, including preserved food, meat, and vegetable groups, were derived (20). The Chinese Food Composition Table was used to derive nutrient indices for each food item and computation of total calories. As another part of the interview, anthropometric measurements were taken, including waist and hip circumferences and height and weight (19). A total of 243 of 264 eligible cases and 472 of 495 eligible controls were interviewed.

Blood Collection and DNA Extraction/Laboratory Analysis. Two-hundred interviewed cases and 330 interviewed controls provided overnight fasting (before 10 a.m.) blood samples for the study. Blood samples were processed and separated within 2 hours of collection at a central laboratory in Shanghai. The blood fractions were stored at −70°C in Shanghai before shipment to the United States on dry ice. DNA was extracted from the buffy coat fractions at the American Type Culture Collection (Manassas, VA). The samples were genotyped at the National Cancer Institute laboratory. Levels of fasting insulin and glucose were measured at F. Stanczyk's laboratory, using commercially available RIA kits (Linco Research, St. Charles, MO). The coefficients of variation of these assays were <8%. The ratio of insulin to glucose was used as an index of insulin resistance (21). For both the genotyping and biomarker assays, laboratory personnel were masked to case-control status in the arrangement of biospecimens.

Molecular Analysis and Assessment of DNA Repair Genes. The selected SNPs were analyzed in 162 cases and 251 controls who had sufficient DNA available using a combination of matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry and Homogeneous MassExtend chemistry (Sequenom, San Diego, CA). SpectroDesigner Software (Sequenom) was used for assay design. Two MALDI-TOF/Homogeneous MassExtend multiplex assays that included the five studied SNPs were selected according to optimum assay partners, reaction terminator mixes, and primers both for genomic PCR amplification of the SNP-containing sequences and DNA polymerase extension within the optimal mass range of 5,000 to 8,500 Da.

The Homogeneous MassExtend procedure, consisting of amplification, cleanup, and extension reactions, was done according to the manufacturer, with some modifications. Desalted PCR and extension oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) and verified by MALDI-TOF mass spectrometry.

For the amplification reaction, the PCR cocktail was composed of 1× AmpliTaq buffer, 2.5 mmol/L $MgCl_2$, 115 mol/L deoxynucleotide triphosphates (Applied Biosystems, Foster City, CA), 3 to 5 ng DNA template, and 0.1 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems). Forward and reverse primers were both at a 100 nmol/L final concentration in a total reaction volume of 6.6 μL. PCR and extension cycling was carried out in an MJ Research thermal cycler (Watertown, MA). PCR conditions included a 15-minute denaturation step at 95°C, followed by 44 cycles of 20 seconds at 95°C, 30 seconds at 62°C and 1 minute at 72°C, followed by a 3-minute hold at 72°C. Amplification was verified by agarose gel electrophoresis. Nonincorporated deoxynucleotide triphosphates were removed by incubating the remaining amplification product at 37°C for 20 minutes in the presence of 0.5 unit shrimp alkaline phosphatase (Sequenom), followed by 5 minutes at 85°C. Extension reactions were carried out by adding 1 to 0.6 mol/L extension primer, 0.5 unit Thermo Sequenase (Sequenom), and a dideoxy/deoxy nucleotide mixture of ddATP, ddCTP, dideoxythymidine 5′-triphosphate, and dGTP (Sequenom) at 50 mol/L each. The following cycling conditions were used: 2 minutes at 95°C, followed by 55 cycles of 5 seconds at 94°C, 5 seconds at 52°C, and 5 seconds at 72°C. Extension products were desalted with SpectroClean Resin (Sequenom). Sequenom 384-element Matrix preloaded Spectro chips were spotted with ~15 nL of sample supernatant with a SpectroJet piezoelectric nanoplotter. MALDI-TOF mass spectrometry was carried out on a Bruker MALDI-TOF Biflex III instrument. Mass spectrometry and genotyping data acquisition were done using a Sequenom SpectroTYPER RT workstation.

The completion rate for genotyping was >98%. Reproducibility of genotyping in 80 duplicate samples from four subjects was >99.9%.

Statistical Analysis. Differences in means of selected characteristics between cases and controls were tested using

t tests, using log-transformation where necessary to meet the normality assumption. Tests for Hardy-Weinberg equilibrium were done using the asymptotic Pearson's χ^2 test. Unconditional logistic regression was used to generate odds ratios (ORs) and 95% confidence intervals (95% CIs) estimating the prostate cancer risk associated with DNA repair markers after adjusting for age and other factors including total caloric intake, waist-to-hip ratio, and insulin resistance (25). We also investigated potential combined effects of the DNA repair polymorphisms with other factors, including waist-to-hip ratio, insulin resistance, and intake of preserved foods and allium vegetables, on prostate cancer risk in this population (19–21), using cutoff levels defined by medians of the distribution of consumption among the control subjects. The significance of interaction terms representing the combined effects of other risk factors with DNA repair markers was tested using the likelihood ratio test (25). All reported *P* values are two-sided.

Results

Selected characteristics of cases and controls are shown in Table 1. Among cases, most tumors were moderately or poorly differentiated, with about two-thirds of the cases displaying clinically significant, advanced cancers in regional or remote stages. The mean waist-to-hip ratio and insulin resistance ratio differed significantly between cases and controls ($P < 0.01$).

Table 1. Selected characteristics for cases and controls

Characteristic	Cases (N = 162) n (%)	Controls (N = 251) n (%)
Education		
No formal education	14 (8.6)	29 (11.6)
Elementary school	58 (35.8)	88 (35.1)
Middle and high school	66 (40.8)	103 (41.0)
College or above	24 (14.8)	30 (12.0)
Other	0	1 (0.3)
Smoking		
Nonsmoker	72 (44.4)	88 (35.1)
Former smoker	42 (25.9)	64 (25.5)
Current smoker	48 (29.6)	99 (39.4)
Alcohol use		
Nondrinker	117 (72.2)	146 (58.2)
Former drinker	17 (10.5)	19 (7.6)
Current drinker	28 (17.3)	86 (34.3)
Hypertension		
No	106 (65.4)	164 (65.3)
Yes	56 (34.6)	87 (34.7)
Diabetes		
No	154 (95.1)	240 (95.6)
Yes	8 (4.9)	11 (4.4)
Prostate cancer stage		
Unknown	1 (0.60)	—
Localized	61 (37.70)	—
Regional	50 (30.90)	—
Remote	48 (29.60)	—
Unstaged	2 (1.20)	—
	Mean (SD)	Mean (SD)
BMI (kg/m^2)	21.75 (2.89)	21.92 (3.16)
Age (y)	72.2 (7.5)	71.7 (7.4)
WHR	0.911 (0.051)	0.889 (0.056)
Total caloric (kcal/d)	2,490 (667)	2,339 (747)
Allium vegetables (g/d)	9.03 (25.12)	15.12 (39.88)
Preserved foods (g/d)	24.43 (30.81)	20.25 (23.46)
PSA (ng/mL)	80.00	

Abbreviations: WHR, waist-to-hip ratio; BMI, body mass index; PSA, prostate-specific antigen.

Table 2. ORs and 95% CIs for prostate cancer in relation to DNA repair gene polymorphisms

DNA repair genes	Cases (N = 162) n (%)	Controls (N = 251) n (%)	OR* (95% CI)
XRCC1-399			
GG	85 (54.8)	132 (54.3)	1.00
AG	53 (34.2)	99 (40.7)	0.83 (0.54-1.28)
AA	17 (11.0)	12 (5.0)	2.18 (0.99-4.81)
AG+AA	70	111	0.97 (0.65-1.46)
XRCC3-241			
CC	139 (87.4)	214 (86.6)	1.00
CT	17 (10.7)	31 (12.6)	0.84 (0.45-1.58)
TT	3 (1.9)	2 (0.8)	2.23 (0.36-13.7)
CT+TT	20	33	0.92 (0.51-1.68)
XPD-751			
AA	141 (88.1)	213 (86.2)	1.00
AC	19 (11.9)	33 (13.4)	0.87 (0.47-1.59)
CC	0	1 (0.4)	—
AC+CC	19	34	0.84 (0.46-1.54)
MGMT-84			
CC	123 (76.4)	213 (86.6)	1.00
CT	36 (22.4)	32 (13.0)	1.95 (1.15-3.30)
TT	2 (1.2)	1 (0.4)	3.39 (0.30-38.1)
CT+TT	38	33	1.99 (1.19-3.34)
MGMT-143			
AA	155 (96.3)	243 (98.0)	1.00
AG	5 (3.1)	5 (2.0)	1.55 (0.44-5.47)
GG	1 (0.6)	0	—
AG+GG	6	5	1.85 (0.55-6.20)

*Adjusted for age.

The genotype distributions among controls were in Hardy-Weinberg equilibrium. Prostate cancer risk estimates associated with the five DNA repair polymorphisms are shown in Table 2. As shown, excess prostate cancer risks were observed for men with the *XRCC1-399* AA, and *MGMT-84* CT/TT genotypes, although the excess was significant only for the *MGMT-84* marker (OR, 1.99; 95% CI, 1.19-3.34).

Table 3 shows the effect of DNA repair polymorphisms on prostate cancer risk in combination with waist-to-hip ratio and insulin resistance. Waist-to-hip ratio was independently associated with an increased risk of prostate cancer in all genotype groups but was more strongly associated with risk among men with the *MGMT-84* CT+TT genotype than among men with the *MGMT-84* CC genotype. Relative to men with the *MGMT-84* CC genotype and lower waist-to-hip ratio, those with a higher waist-to-hip ratio (waist-to-hip ratio $\geq$0.892) and harboring the *MGMT-84* CT+TT genotype had an almost 4-fold increased risk (OR, 3.64; 95% CI, 1.74-7.62). However, there was no significant interaction between the *MGMT-84* polymorphism and waist-to-hip ratio. In contrast, a significant interaction ($P_{\text{interaction}} = 0.046$) was observed between the *MGMT-84* marker and insulin resistance. Insulin resistance was associated with an increased risk of prostate cancer, regardless of genotype. Relative to those with the low-risk genotype (CC) and a lower insulin resistance, men with the high-risk *MGMT-84* CT+TT genotype and a greater insulin resistance (>0.097) had the highest risk (OR, 5.39; 95% CI, 2.46-11.82).

Table 4 shows the ORs and 95% CIs for prostate cancer in relation to DNA repair gene polymorphisms by consumption of preserved food and allium vegetables. A significant interaction was found for *XRCC3-241* and consumption of total preserved foods. Those with the *XRCC3-241* CT+TT genotype and a higher consumption of total preserved foods ($\geq$12.7 g/d) had a 2.6-fold risk (95% CI, 1.13-6.06), whereas those with the CT+TT genotype and a low intake of total preserved foods had a reduced risk (OR, 0.31; 95% CI, 0.10-0.96; $P_{\text{interaction}} = 0.005$). Similar risk patterns were seen for consumption of individual preserved foods, including

Table 3. ORs and 95% CIs for prostate cancer in relation to DNA repair gene polymorphisms, by waist-to-hip ratio and insulin resistance

DNA repair gene	WHR*	Cases (N = 162)	Controls (N = 251)	OR[†] (95% CI)	$P_{interaction}$[‡]
XRCC1-399					
GG	<0.892	26	67	1.00	0.788
AG+AA	<0.892	22	56	0.96 (0.48-1.92)	
GG	>0.892	58	65	1.85 (1.02-3.36)	
AG+AA	≥0.892	47	55	1.57 (0.84-2.94)	
XRCC3-241					
CC	<0.892	42	109	1.00	0.595
CT+TT	<0.892	7	16	1.29 (0.49-3.40)	
CC	≥0.892	95	105	1.83 (1.14-2.94)	
CT+TT	≥0.892	13	17	1.67 (0.72-3.88)	
XPD-751					
AA	<0.892	42	104	1.00	0.872
AC+CC	<0.892	6	20	0.84 (0.30-2.29)	
AA	≥0.892	97	109	1.76 (1.10-2.83)	
AC+CC	≥0.892	13	14	1.65 (0.67-4.06)	
MGMT-84					
CC	<0.892	42	107	1.00	0.101
CT+TT	<0.892	7	17	0.92 (0.34-2.46)	
CC	≥0.892	80	106	1.46 (0.90-2.38)	
CT+TT	≥0.892	30	16	3.64 (1.74-7.62)	
MGMT-143					
AA	<0.892	48	123	1.00	0.938
AG+GG	<0.892	1	2	1.11 (0.09-14.02)	
AA	≥0.892	106	120	1.76 (1.13-2.75)	
AG+GG	≥0.892	4	3	2.19 (0.41-11.70)	
DNA repair gene	Insulin resistance*	Cases (N = 162)	Controls (N = 251)	OR[§] (95% CI)	$P_{interaction}$[‖]
XRCC1-399					
GG	<0.097	28	61	1.00	0.473
AG+AA	<0.097	19	57	0.75 (0.37-1.50)	
GG	≥0.097	56	68	1.78 (0.99-3.22)	
AG+AA	≥0.097	50	53	1.83 (0.99-3.39)	
XRCC3-241					
CC	<0.097	40	104	1.00	0.560
CT+TT	<0.097	8	18	1.15 (0.46-2.87)	
CC	≥0.097	97	106	2.26 (1.40-3.65)	
CT+TT	≥0.097	12	15	1.79 (0.75-4.26)	
XPD-751					
AA	<0.097	43	103	1.00	0.326
AC+CC	<0.097	4	18	0.57 (0.18-1.79)	
AA	≥0.097	96	108	2.03 (1.27-3.24)	
AC+CC	≥0.097	15	14	2.28 (0.99-5.25)	
MGMT-84					
CC	<0.097	40	100	1.00	0.046
CT+TT	<0.097	8	21	0.94 (0.38-2.32)	
CC	≥0.097	82	109	1.79 (1.10-2.92)	
CT+TT	≥0.097	29	12	5.39 (2.46-11.82)	
MGMT-143					
AA	<0.097	45	121	1.00	0.160
AG+GG	<0.097	3	1	4.34 (0.37-50.83)	
AA	≥0.097	109	118	2.29 (1.46-3.59)	
AG+GG	≥0.097	2	4	1.23 (0.21-7.10)	

*Median among controls used as the cutoff.
†Adjusted for age and insulin resistance.
‡P value for interaction between marker and waist-to-hip ratio, adjusted for insulin resistance and age.
§Adjusted for age and waist-to-hip ratio.
‖P value for interaction between marker and insulin resistance, adjusted for waist-to-hip ratio and age.

preserved animal foods, preserved vegetables, and preserved eggs (data not shown). No combined effects with allium vegetable intake were observed for any of the studied DNA repair markers.

Discussion

In this population-based study, we found that polymorphisms of genes in DNA repair pathways, including *XRCC1-399* and the *MGMT-84*, are independently associated with prostate cancer risk. No significant independent associations with prostate cancer were observed for the *XRCC3-241, XPD-751*, or *MGMT-143* markers, although small to modest effects cannot be ruled out because of small sample size. We also found that men who have these susceptibility markers in conjunction with a higher waist-to-hip ratio, greater insulin resistance, or higher intake of preserved foods had a much higher risk of prostate cancer.

The *XRCC1* gene product is involved in the base excision repair pathway, which corrects DNA damage primarily from ionizing radiation and alkylating agents (12, 26). Few studies

Table 4. ORs and 95% CIs for prostate cancer in relation to DNA repair gene polymorphisms and intake of total preserved foods and allium vegetables

DNA repair gene	Preserved food (g/d)*	Cases (N = 162)	Controls (N = 251)	OR† (95% CI)	$P_{interaction}$‡
XRCC1-399					
GG	<12.7	34	73	1.00	0.463
AG+AA	<12.7	26	50	1.08 (0.58-2.03)	
GG	≥12.7	51	59	1.79 (1.02-3.15)	
AG+AA	≥12.7	44	61	1.48 (0.84-2.61)	
XRCC3-241					
CC	<12.7	58	103	1.00	0.005
CT+TT	<12.7	4	22	0.31 (0.10-0.96)	
CC	≥12.7	81	111	1.25 (0.81-1.93)	
CT+TT	≥12.7	16	11	2.62 (1.13-6.06)	
XPD-751					
AA	<12.7	54	106	1.00	1.00
AC+CC	<12.7	8	18	0.86 (0.35-2.12)	
AA	≥12.7	87	107	1.54 (0.99-2.38)	
AC+CC	≥12.7	11	16	1.36 (0.58-3.15)	
MGMT-84					
CC	<12.7	46	107	1.00	0.641
CT+TT	<12.7	17	17	2.31 (1.08-4.95)	
CC	≥12.7	77	106	1.66 (1.05-2.63)	
CT+TT	≥12.7	21	16	2.81 (1.33-5.94)	
MGMT-143					
AA	<12.7	61	121	1.00	0.219
AG+GG	<12.7	2	4	0.93 (0.16-5.27)	
AA	≥12.7	94	122	1.48 (0.98-2.25)	
AG+GG	≥12.7	4	1	7.53 (0.81-70.2)	
DNA repair gene	Allium vegetables (g/d)*	Cases (N = 162)	Controls (N = 251)	OR† (95% CI)	$P_{interaction}$‡
XRCC1-399					
GG	<5.83	54	66	1.00	0.300
AG+AA	<5.83	51	55	1.08 (0.64-1.84)	
GG	≥5.83	31	66	0.53 (0.30-0.94)	
AG+AA	≥5.83	19	56	0.39 (0.21-0.74)	
XRCC3-241					
CC	<5.83	94	107	1.00	0.772
CT+TT	<5.83	13	17	0.88 (0.40-1.93)	
CC	≥5.83	45	107	0.45 (0.29-0.71)	
CT+TT	≥5.83	7	16	0.50 (0.20-1.28)	
XPD-751					
AA	<5.83	93	105	1.00	0.738
AC+CC	<5.83	14	18	0.88 (0.41-1.88)	
AA	≥5.83	48	108	0.47 (0.30-0.74)	
AC+CC	≥5.83	5	16	0.35 (0.12-0.99)	
MGMT-84					
CC	<5.83	79	107	1.00	0.249
CT+TT	<5.83	29	16	2.34 (1.18-4.63)	
CC	≥5.83	44	106	0.54 (0.34-0.85)	
CT+TT	≥5.83	9	17	0.63 (0.26-1.54)	
MGMT-143					
AA	<5.83	103	122	1.00	0.339
AG+GG	<5.83	5	2	2.93 (0.55-15.7)	
AA	≥5.83	52	121	0.48 (0.32-0.74)	
AG+GG	≥5.83	1	3	0.33 0.03-3.32)	

*Total preserved foods includes preserved meats, eggs, and vegetables. Allium vegetables include onions, leeks, garlic, chives, and scallions. Median intake among controls used as the cutoff.
†Adjusted for age and total caloric intake.
‡P value for interaction between marker and either preserved foods or allium vegetable intake, adjusted for age and total caloric intake.

have investigated the role of *XRCC1* in prostate cancer. Previous studies in U.S. men have found nonsignificantly increased prostate cancer risks associated with the AA genotype (27, 28). Consistent with these previous observations, in this Shanghai population, we found a borderline significant independent risk increase associated with the *XRCC1*-399 AA genotype.

To our knowledge, no epidemiologic studies have investigated the role of *MGMT-84* and prostate cancer risk. Our results suggest that the *MGMT-84* T variant (the high-risk allele) may have a dominant effect, although we were unable to evaluate the effect of the TT genotype due to small numbers. We previously reported strong associations of abdominal obesity and insulin resistance with prostate cancer risk in this Shanghai population (19, 21, 29). In the current study, we show further that the excess prostate cancer risks associated with these risk factors are more pronounced among men carrying the variant allele of the *MGMT-84* marker. Both obesity and insulin resistance are closely linked to chronic inflammation, which in turn can cause DNA damage (30, 31). The product of the *MGMT* gene (also known as the O^6-alkylguanine-DNA alkyltransferase or AGT) operates through the direct DNA damage reversal pathway, repairing DNA damage caused by alkylating agents (14, 17), and is susceptible to inactivation or degradation by oxidative agents (32). In

addition, hypermethylated *MGMT* can be found in prostate carcinomas and is believed to be involved in prostate carcinogenesis (33). Although of interest, the observed joint effects of *MGMT-84* with insulin resistance need to be confirmed in larger studies.

The combined effect of the *XRCC3-241* marker and intake of preserved foods was intriguing. Despite our observation of no independent effect of the *XRCC3-241* polymorphism on prostate cancer risk, higher intake of preserved foods was associated with a modest 25% increase in prostate cancer risk among men with the CC genotype of the marker. In contrast, among men with the CT/TT genotypes, higher preserved food intake was associated with a markedly increased risk. Consumption of salted and preserved foods is relatively common in China and is a risk factor for prostate cancer in this study. These foods are abundant in nitrosamines and nitrosamine precursors, which are considered important food mutagens (34–36) and can transform adult human prostate epithelial cells (37). The *XRCC3* gene product participates in DNA double-strand break/homologous recombination repair. *In vitro* studies suggest that homologous recombination repair enzymes, including the *XRCC3* gene product, may be involved in resistance to alkylating DNA damage induced by nitrogen mustards (22, 23). The observed significant joint effects of preserved foods with the *XRCC3-241* marker suggest a potential gene-environment effect that warrants further investigation.

Due to the small number of SNPs studied per gene in this investigation, it is possible that the observed associations are due to linkage disequilibrium of the studied loci with other nearby SNPs within the studied genes. Similarly, other genes, including those in other DNA damage repair pathways, may be related to the SNPs examined in this study and may have confounded the results. Expanded studies with large sample sizes and more comprehensive coverage of SNPs in these and other genes in the DNA repair pathways are needed to evaluate the effect of gene-gene and gene-environment interactions.

Survival and selection biases are minimal in this population-based study: Over 90% of the incident cases in Shanghai during the study period agreed to be interviewed for the study, and demographic and clinical factors did not differ between interviewed subjects who did and did not participate in the blood collection. In addition, misclassification of genotype is minimal in the study, as evidenced by the 99.9% concordance of genotyping results in 80 duplicate quality control samples. However, for certain markers, statistical power is limited due to the rarity of the variant alleles, and for that reason we cannot rule out the possibility of small-to-modest effects of these markers, particularly *MGMT-143*. Our assessment of SNP-covariate interactions in this study is limited by low statistical power (<50%). Therefore, we may have been unable to detect modest true interactions. Our results for interactions of DNA repair markers with other factors in this study should be considered merely suggestive, but they may serve to indicate new areas of research, particularly exposure-specific functional studies of these SNPs. Finally, because the population in Shanghai is ethnically homogeneous, our results may not be generalizable to other populations.

In summary, this population-based study conducted in a low-risk population suggests that the DNA repair polymorphisms, in particular *XRCC1-399*, *XRCC3-241*, and *MGMT-84*, may be associated with the risk of clinically significant prostate cancer in China. Our results also suggest that other risk factors, including preserved food intake, insulin resistance, and abdominal obesity, may operate in concert with genes in the DNA repair pathways to influence risk, although these findings from subgroup analyses should be considered suggestive and must be confirmed. Although we are unable to generalize our results directly to other populations, similar underlying biological mechanisms may exist for other racial/ethic groups. Larger studies are needed to confirm these results and clarify the mechanisms involved.

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